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Research paper

Plasmid Profile of Multiple Antibiotics Resistant (mar) *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* Isolated from Water Samples from Ebira Communities in Ekiti South Senatorial District

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Abstract

Plasmid curing of microbes and physicochemical analysis of water samples obtained from Ebira communities in six local governments in Ekiti South Senatorial District were analyzed. Antibiotic sensitivity and profile of bacterial isolates were analyzed using pour plating, disk diffusion method and gel electrophoresis techniques respectively while the plasmid were cured using acridine orange. The mean total bacterial count of the water samples collected from these six different local governments at different time ranged from 2.08 x 10^5 to 6.0 x 10^6 CFU/ml; the mean total coliform count ranged from 2.41 x 10^5 to 3.75 x 10^6 CFU/ml and the mean total *Escherichia coli* count (TEC) ranged from 1.53 x 10^5 to 3.45 x 10^5 CFU/ml. Total of 152 bacteria were recovered with *E.coli* having the highest distribution of 35% while *Serratia marcensens* had the least distribution of 0.7%. The highest antibiotic resistance of 100% was recorded against ceftazidine but only 17% of the isolates were resistant to gentamicin. About 56% of 34 selected MAR isolates carried plasmid(s) with high molecular weight ranging from 5.64Kbp to 23.13Kbp. Antibiotic resistance pattern and plasmids profile of selected MAR *E.coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* prior to and after curing showed that *Pseudomonas aeruginosa* became susceptible to augmentin and *Staphylococcus aureus* also became susceptible to ceftriazole while *E. coli* still maintained the earlier resistant pattern. The plasmid profiling of these isolates after curing indicated the lost of plasmids in each of the isolates. Present study however implicated the incidence of MAR bacteria in the sources of water in Ekiti-South Senatorial district as a serious health challenge, and confirmed the potential of acridine orange for plasmid curing.

Keywords: *Plasmid Curing; Ekiti South Senatorial District; MAR; Acridine Orange.*

1. Introduction

With growing populations and an overall increase in living standards, not only is the overall demand for freshwater pushing limits, but increasing pollution from urban, industrial, and agricultural sources is also making available resources either unusable or dangerous to health (Figueras and Borrego, 2010). Water stress occurs when the demand for water exceeds the available amount during a certain period or when poor quality restricts its use. Water stress causes deterioration of freshwater resources in terms of quantity (aquifer over-exploitation, dry rivers, etc.) and quality (eutrophication, organic matter pollution, saline intrusion etc.).
Every human needs about 20 litres of freshwater a day for basic survival (drinking and cooking) and an additional 50 to 150 litres for basic household use. Rural communities around the world traditionally take their water supply from rivers or from shallow dug wells. Growing concentrations of people combined with the increasing industrialization of land use have resulted in many major rivers becoming highly polluted. Key pollutants in the water systems are typically pathogens arising from human waste (bacteria and viruses), heavy metals, and organic chemicals from industrial waste. Water pollution is one of the greatest causes of mortality that can be linked to environmental factors. Almost five million deaths in the developing world annually are due to water-related diseases.
This research focused on incidence of MAR bacteria among the microbial isolates from the leachate contaminated water sources from Ebira communities in Ekiti South Senatorial district of Ekiti State, Nigeria. In addition potency of acridine orange as plasmid curing agent would be evaluated.

2. Materials and methods

2.1. Sampling sites

Water samples were collected from various communities in six local government areas from Ekiti South Senatorial district in Ekiti State comprising Ekiti East, Ekiti South West, Emure, Gbonyin, Ikere and Ise/Orun. The sampling points are: Aba Ola, Aba Afolu, Aba Oyo, Eporo, Ijaloke, Aba Isua, Kajola, Araromi, Ikere Ekiti, Aisegba Ekiti, Ogotun Ekiti, Ijan Ekiti, Ilumoba Ekiti, Igbara-odo Ekiti, Edugbe, Erinta, Iworo.

2.2. Collection of water samples

Water samples were collected aseptically in 250ml sterile sampling bottles and they were collected from three different sources; well (W), stream (S), borehole (B). The samples were transported in ice-bag to the Microbiology Laboratory of Ekiti State University, Ado-Ekiti and were subjected to immediate analysis within two hours of sample collection.

2.3. Microbiological analysis

The water samples were analyzed on different culture media using the standard pour plate technique. Nutrient agar was used for the heterotrophic plate count; Eosin Methylene Blue (EMB) for the isolation of *Escherichia coli*, mannitol salt agar (MSA), for the isolation of *Staphylococcus aureus*, MacConkey agar for *Enterobacter* and other coliforms (APHA, 1998). Colonies with distinct characteristics on each culture medium were identified on the basis of their morphological, sugar fermentation and biochemical properties using the scheme in the Bergey's manual of Determinative Bacteriology (Holt *et al*., 1994).

2.3.1. Antibiotic susceptibility test

Inocula for standard antimicrobial susceptibility tests were prepared by touching four to five similar colonies of Gram-positive or Gram-negative bacteria with a loop, transferring these colonies to tryptic soy broth, and incubating them at 35°C for 2 to 5h until the turbidity was equivalent to a 0.5 $BaSO_4$ standard. Suspensions in tryptic soy broth equivalent to a $BaSO_4$ standard were prepared from a 24h culture plate of the fastidious organisms.
Colony counts were performed on each inoculum by subculturing various dilutions of the inocula in water. A 0.1ml volume of each dilution was subcultured onto an appropriate agar plate, spread with a sterile glass rod, incubated for 24 to 48h at 35°C, and counted with a Fisher bacterial colony counter, model 480.
Antibiotic susceptibility was done using the disc diffusion method for each of the isolate as described by Cheesbrough (2006). The antibiotics used were; cefotaxime (CAZ 3µg), cefuroxime (CRX 30µg), gentamycin (GEN 10µg,), augmentin (Aug 30µg,) amoxillin (AMX 30µg), nitrofurantoin (NIT 30µg) ceftaxidine (CTX 30µg), ofloxacin (OFL 5µg). The diameter of zone of the clearance including the diameter of the disk was measured to the nearest whole millimeter and interpreted on the basis of CLSI (2005) guidelines.

2.3.2. Isolation of plasmid DNA and agarose gel electrophoresis

The multiple antibiotics resistant (MAR) isolates of Salmonella species and the antibiotic sensitive Salmonella species isolates were subjected to plasmid DNA isolation according to the protocol of Birnboim and Doly (1979); Kado and Liu (1981) with some modifications. Agarose gel electrophoresis of the isolated plasmid DNA was carried out in tris-borate buffer system, using 1.2% agarose, for 1 h at 75v.

a) The plasmids DNA were isolated using lysing solution.
The lysate were kept in ice for 30 min and centrifuged for 5 min, phenol: chloroform (1:1) treatment was followed with the clear supernatant. Plasmid DNA were precipitated with equal volume of chilled isopropyl alcohol and DNA pellet dissolved in 100 µl of TE buffer (diethylether).

b) A 0.8% agarose gel was used to resolve DNA fragment and it was prepared by combining 0.8 g agarose in ten times concentration of Tris acetate ethylene diamine tetraacetate (10 ml 10XTAE) buffer and 90 ml distilled water in a 250 ml beaker flask and heating in a microwave for 2 min until the agarose is dissolved. 2.5 ml ethidium bromide (5.0 mg/ml) was added to the dissolved agarose solution with swirling to mix. The gel was then poured onto a mini horizontal gel electrophoresis tank and the casting combs were inserted. It was then allowed to gel for 30 min. The casting comb was then carefully removed after the gel had completely solidified. One times concentration (IX) TAE electrophoresis buffer was then added to the reservoir until the buffer just covered the agarose gel. 0.5 µl of gel tracking dye (bromophenol blue) was added to 20 µl of each sample with gentle mixing. 20 µl of the sample was then loaded onto the wells of the gel, the mini horizontal electrophoresis gel set-up was then covered and the electrodes connected. Electrophoresis was carried out at 100 - 120 mA for 1 h. At the completion of the electrophoresis, the gel was removed from the buffer and gel was viewed under a long wave UV-light box. The band pattern of the DNA fragments were then photographed with a Polaroid camera and documented using an electrophoresis gel documentation system. The molecular sizes of each plasmid were determined by comparison with plasmids of known mass (Datta *et al*., 1971).

2.3.3. Plasmid curing

Three of the MAR isolates, *E. coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were selected for the curing of antibiotic resistance plasmids. The plasmids were cured by treatment with acridine orange according to the method of Brown (2000). Buffered Peptone broth was prepared and supplemented with 0.1mg/ml acridine orange. 20µl of overnight culture of the bacteria was sub cultured into 5mls of the nutrient broth containing acridine orange. The samples were then incubated at 37°C for 72hours. After 72 hours of incubation, the isolates were swabbed to the Mueller Hinton agar plates and plasmid extraction was repeated on some of the organisms to verify if the plasmid were successfully cured.

2.4. Physicochemical analysis

The Water samples were immediately brought into Laboratory for the Estimation of various physicochemical parameters. Temperature (°C) and pH, turbidity (JTU), electrical conductivity (µmho/cm) were recorded at the time of sample collection by using Thermometer and Pocket Digital pH Meter. While other Parameters Such as DO, Hardness, Alkalinity, Chlorides, Phosphate, Nitrate, magnesium (mg/l), and sulphate (mg/l) were estimated in the Laboratory by using Indian Standard Procedures (Titration method, Atomic Absorption Spectrophotometer (AAS) Thermo M5 Model) (Trivedy and Goel,1986; APHA, 1985).

3. Results

The total bacterial, total coliform and total enteric bacteria counts of the water samples from six different local governments are depicted in Table 1. The mean TBC value ranges from 2.08×10^5 CFU/ml to 6.0×10^6 CFU/ml; the mean TCC value ranges from 2.41×10^5 CFU/ml to 3.75×10^6 CFU/ml while the mean TEC value ranges from 1.53×10^5 CFU/ml to 3.45×10^5 CFU/ml.
The percentages distribution of isolated bacteria revealed *Escherichia coli* with the highest percentage (35%), followed by *Staphylococcus aureus* (6.6%), *Enterococcus feacalis* (8.6%), *Klebsiella* spp (28.9%), *Enterobacter aerogenes* (7.23%), *Pseudomonas aerogenes* (1.97%), *Proteus vulgaris* (2.63%), *Bacillus cereus* (7.9%) and *Serratia marcenscens* (0.7%) with the least percentage distributions (Table 2).
All sixty nine (100%) Gram positive bacteria isolated from water samples were highly resistant to ceftazidime and 13% were resistant to ofloxacin (Table 3). Meanwhile, 88% of eighty three (83) Gram negative bacteria isolated from the water samples were highly resistant to cefuroxime and 17% were resistant to gentamicin (Table 4).
Multiple antibiotics resistance (MAR) is higher among Gram negative isolates than Gram positive isolates (Table 5). Eighteen (18) out of thirty four (34) isolates selected, possessed single plasmid except *E.coli*29 which had 2 plasmids while the remaining 15 isolate had no plasmid with molecular weight ranging from

5.64Kbp to 23.13Kbp. The plasmid pictorial representation of the Gram positive and Gram negative isolates is depicted in figure 1 and figure 2 respectively.

Table 1: Microbial Estimation (CFU/Ml) of Water Samples

Water samples	Total Bacteria Count		Total Coliform Count		Total Enterococcus Count	
	10^5	10^6	10^5	10^6	10^5	10^6
AOB	0.50	0.12	0.09	0.00	0.03	0.00
AOW	0.22	0.13	0.25	0.06	0.00	0.00
AOS	0.85	0.20	0.55	0.25	0.60	0.42
MEAN VALUE	0.52	0.15	0.30	0.10	0.21	0.14
BAB	0.09	0.00	0.07	0.00	0.09	0.03
BAW	0.55	0.20	0.40	0.20	0.10	0.07
BAS	3.20	1.49	0.75	0.30	0.27	0.18
MEAN VALUE	1.28	0.56	0.41	0.16	0.15	0.09
CAB	0.24	0.15	0.14	0.06	1.39	0.27
CAW	0.44	0.28	0.24	0.14	0.60	0.43
CAS	2.08	5.20	2.41	2.41	1.53	2.80
MEAN VALUE	0.92	1.88	2.63	0.87	2.59	3.32
DEB	0.05	0.00	0.08	0.02	0.08	0.00
DEW	0.64	0.30	0.13	0.00	0.60	0.03
DES	2.82	1.59	0.24	0.05	1.38	1.11
MEAN VALUE	1.17	0.63	0.15	0.02	2.06	0.38
EIB	0.07	0.05	0.80	0.30	0.07	0.00
EIW	0.50	0.21	0.80	0.30	0.48	0.14
EIS	2.00	1.49	2.00	1.41	1.34	1.24
MEAN VALUE	0.86	0.58	3.07	0.67	0.63	0.46
FAB	0.00	0.00	0.00	0.00	0.00	0.00
FAW	0.21	0.13	0.08	0.02	0.17	0.60
FAS	0.04	6.00	0.04	0.02	0.00	0.00
MEAN VALUE	0.08	2.04	0.04	0.01	0.06	0.20
GKB	0.06	0.00	0.05	0.02	0.04	0.00
GKW	1.56	1.00	1.26	1.22	1.80	1.10
GKS	0.26	0.05	0.55	0.00	0.10	0.00
MEAN VALUE	0.63	0.35	0.62	0.41	0.65	0.37
HAB	0.00	0.00	0.00	0.00	0.00	0.00
HAW	0.12	0.07	0.00	0.00	0.35	0.21
HAS	0.08	0.05	0.00	0.00	0.08	0.00
MEAN VALUE	0.07	0.04	0.00	0.00	0.14	0.07
IKB	0.55	0.25	0.00	0.00	0.05	0.49
IKW	2.05	1.22	1.00	0.00	0.65	0.58
IKS	2.02	1.42	1.50	1.00	1.45	3.45
MEAN VALUE	1.54	0.96	0.83	0.33	1.72	2.22
JAB	0.50	0.08	0.15	0.00	0.35	0.00
JAW	0.33	0.10	0.24	0.12	0.15	0.08
JAS	2.01	0.68	0.49	0.00	1.39	0.00
MEAN VALUE	0.95	0.29	0.29	0.04	0.63	0.03
KOB	0.53	0.03	0.55	0.22	0.30	0.00
KOW	2.08	2.08	2.08	1.08	0.05	0.00
KOS	0.30	0.50	0.75	1.52	1.35	0.00
MEAN VALUE	0.97	0.87	2.88	0.94	0.56	0.00
LIB	0.03	0.00	0.05	0.00	0.06	0.00
LIW	0.03	0.00	0.02	0.01	0.15	0.08
LIS	0.22	0.11	1.28	0.45	0.00	0.00
MEAN VALUE	0.09	0.04	0.45	0.15	0.07	0.02
MIB	0.04	0.00	0.00	0.00	0.12	0.10
MIW	0.52	0.50	0.30	0.00	0.40	0.17
MIS	0.76	0.45	0.60	0.22	0.35	0.10
MEAN VALUE	0.44	0.32	0.30	0.07	0.36	0.12
NIB	0.03	0.00	0.04	0.00	0.04	0.00
NIW	0.03	0.00	0.00	0.00	0.15	0.06
NIS	0.51	0.20	0.08	0.04	0.00	0.00
MEAN VALUE	0.19	0.06	0.02	0.01	0.06	0.02
OEB	0.04	0.00	0.00	0.00	0.01	0.00
OEW	1.24	0.92	0.60	0.20	1.02	1.22
OES	1.80	1.00	2.38	3.75	1.00	1.01
MEAN VALUE	1.03	0.64	0.99	1.45	2.11	0.74
PEB	0.00	0.00	0.00	0.00	0.00	0.00
PEW	0.07	0.00	0.00	0.00	0.15	0.08
PES	0.12	0.07	0.00	0.00	0.35	0.20
MEAN VALUE	0.06	0.02	0.00	0.00	0.17	0.93
QIB	0.20	0.04	0.06	0.00	0.00	0.00
QIW	0.23	0.00	0.73	0.00	0.30	0.05
QIS	0.50	0.10	0.25	0.10	0.15	0.00
MEAN VALUE	0.31	0.05	0.35	0.03	0.15	0.02

KEYS:

AOB– Aba Ola Bore Hole	AOW- Aba Ola Well	AOS- Aba Ola Stream
BAB– Afo Olu Bore Hole	BAW-Afo Olu Well	BAS- Afo Olu Stream
CAB– Aba Oyo Bore Hole	CAW- Aba Oyo Well	CAS- AbaOyo Stream
DEB– Eporo Bore Hole	DEW- Eporo Well,	DES- Eporo Stream
EIB– Ija Loke Bore Hole	EIW- Ija Loke Well	EIS- Ija Loke Stream
FAB– Aba Isua Bore Hole	FAW- Aba Isua Well	FAS- Aba Isua Stream
GKB– Kajola Bore Hole	GKW- Kajola Well	GKS- Kajola Stream
HAB– Araromi Bore Hole	HAW-Araromi Well	HAS- Araromi Stream
IKB–Oke-Ikere Bore Hole	IKW- Oke-Ikere Well	IOS- Oke Ikere Stream
JAB– Aisegba Bore Hole	JAW- Aisegba well	JAS- Aisegba stream
KOB– Ogotun Bore Hole	KOW- Ogotun Well	KOS- Ogotun Stream
LIB– Ijan Bore Hole	LIW- Ijan Well	LIS- Ijan Stream
MIB– Ilumoba Bore Hole	MIW-Ilumoba Well	MIS- Ilumoba Stream
NIB– Igbara Odo Bore Hole	NIW- Igbara Odo Well	NIS- Igbara Odo Stream
OEB– Edugbe Bore Hole	OEW- Edugbe Well	OES- Edugba Stream
PEB– Erita Bore Hole	PEW- Erita Well	PES- Erita Stream
QIB– Iworo Bore Hole	QIW-Iworo Well	Q IS- Iworo Stream

Table 2: Distribution of Organisms Isolated from Different Sampling Points

Isolates	Distribution																	Number of isolate	Frequency %
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q		
Escherichia coli	4	3	4	3	1	3	2	2	4	2	7	10	7	10	3	6	2	54	35.5
Staphylococcus aureus	2	-	-	-	4	-	-	-	2	-	2	-	1	-	1	-	1	13	8.6
Enterococcus faecalis	3	5	-	2	1	2	4	2	-	2	4	1	-	7	-	6	5	44	28.9
Enterobacter aerogenes	1	-	-	4	-	2	-	2	-	-	1	-	-	1	-	-	-	10	6.6
Klebsiella spp	-	-	-	2	-	-	1	-	2	-	-	3	-	-	1	1	-	11	7.2
Pseudomonas aeruginosa	1	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	3	2.0
Proteus vulgaris	2	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	4	2.6
Bacillus cereus	4	2	1	-	-	-	-	1	-	-	1	-	-	-	-	1	2	12	7.9
Serratia marcescens	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.7
Total																		152	100

KEYS:

A- Aba Ola, B- Afo Olu, C- Aba Oyo, D- Eporo, E- Ija Loke, F- Aba Isua, G- Kajola, H- Araromi, I- Oke-Ikere, J-Aisegba, K-Ogotun, L-Ijan, M-Ilumoba, N-Igbara-Odo, O-Edugbe, P- Erita, Q-Iworo.

Table 3: Antibiotics Resistance Pattern of Isolated Gram Negative Bacteria

Test Organisms	CAZ	CRX	GEN	CTX	OFL	AUG	NIT	AMX	Phenotype of Resistance Pattern
Escherichia coli									
1	R	R	R	S	S	R	S	I	CAZ, CRX, GEN, AUG
2	R	R	R	S	S	R	S	R	CAZ, CRX, GEN, AUG,AMX
3	R	S	S	R	S	R	R	I	CAZ, CTX, AUG,NIT
4	R	R	S	R	S	R	R	R	CAZ,CRX,CTX,AUG,AMX
5	R	S	I	R	S	R	R	R	CAZ,CXM,AUG,NIT,AMX
6	R	R	S	R	S	R	S	S	CAZ, CRX, CTX, AUG
7	I	I	I	R	S	R	R	R	CXM, AUG, NIT, CPR
8	R	R	I	S	R	R	S	R	CAZ, CRX, OFL, AUG, CPR
9	R	R	S	R	S	R	R	R	CAZ, CRX, CTX, AUG, NIT,AMX
10	R	R	S	R	S	R	S	R	CAZ, CRX, CTX, AUG, CPR
11	R	R	S	R	S	R	S	S	CAZ, CRX, CXM, AUG
12	R	R	S	S	S	R	S	R	CAZ, CRX,AUG,AMX
13	R	R	R	S	S	R	S	R	CAZ, CRX, GEN, AUG, AMX
14	R	R	R	S	R	R	S	S	CAZ, CRX, GEN, OFL, AUG
15	R	R	S	R	S	R	S	R	CAZ, CRX, CTX, AUG, AMX
16	R	R	S	R	S	S	R	R	CAZ, CRX, CTX, AUG, AMX
17	R	R	S	R	S	R	R	R	CAZ,CRX,CTX,AUG,NIT,AMX
18	R	R	S	R	R	R	I	R	CAZ,CRX,CTX,OFL,AUG,AMX
19	R	R	S	R	S	R	S	R	CAZ,CRX,CTX,OFL,AUG,NIT,AMX
20	R	R	R	I	S	R	S	R	CAZ,CRX,GEN, AUG, AMX
21	R	R	S	R	S	R	S	S	CAZ,CRX,CXM, AUG
22	R	R	S	R	S	S	S	R	CAZ, CRX, CXM, AMX
23	I	R	S	R	S	R	R	R	CRX,CTX,AUG,NIT,AMX
24	S	R	S	R	S	R	S	R	CRX, CXM, AUG, AMX
25	R	R	S	R	S	R	S	S	CAZ,GEN,CTX,OFL,AUG,NIT,AMX
26	R	S	S	R	S	R	R	S	CAZ, CTX,AUG,NIT
27	I	R	S	R	S	R	R	R	CRX, CXM, AUG, NIT, AMX
28	R	R	S	R	S	S	R	R	CAZ, CRX, CTX,NIT,AMX
29	S	R	S	R	S	S	R	R	CRX, CTX, NIT, AMX
30	R	R	S	R	S	R	I	S	CAZ, CRX, CXM, AUG.
31	R	R	S	R	I	R	S	S	CAZ, CRX, CXM, AUG.
32	R	R	S	R	S	R	R	S	CAZ, CRX, CXM, AUG, NIT
33	R	S	R	S	S	R	R	S	CAZ,GEN,AUG, NIT
% Resistance of Antibiotics	88%	84%	21%	76%	12%	82%	39%	58%	
Klebsiella spp									
1	R	R	R	S	S	R	S	S	CAZ, CRX, GEN, AUG
2	R	R	R	S	S	R	I	R	CAZ, CRX, GEN, AUG, AMX.
3	R	R	S	R	S	R	R	R	CAZ,CRX,CXM,AUG,NIT,AMX
4	I	S	S	R	S	R	R	R	CXM, AUG, NIT, AMX
5	R	R	S	R	S	R	R	S	CAZ, CRX, CXM, AUG,NIT
6	R	R	S	R	S	R	R	S	CAZ, CRX, CXM, OFL,AUG
7	R	R	S	R	R	R	R	S	CAZ,CRX,CXM,OFL,AUG,NIT
% Resistance to Antibiotics	85%	85%	28%	71%	14%	100%	71%	42%	
Enterobacter aerogenes									
1	S	R	R	R	S	R	S	S	CRX, GEN, CXM, AUG
2	S	R	R	R	S	R	R	S	CRX,GEN, CXM, AUG, NIT
3	S	R	S	R	S	R	R	R	CRX, CXM, AUG, NIT,AMX
4	S	S	S	R	S	R	R	R	CXM, AUG, NIT, CPR
5	R	R	R	S	I	R	I	S	CAZ, CRX, GEN, AUG
6	R	S	S	R	I	R	S	R	CAZ, CXM, AUG, AMX
7	S	R	S	R	S	R	R	R	CRX,CXM, AUG, NIT, AMX
8	R	R	S	R	S	R	S	R	CAZ, CRX, CXM,AUG,AMX
9	R	R	S	R	S	R	S	S	CAZ, CRX, CXM, AUG
10	R	R	S	R	S	R	R	S	CAZ, CRX, CXM, AUG, NIT
% Resistance to Antibiotics	50%	80%	30%	90%	0%	100%	50%	50%	
Pseudomonas aeruginosa									
1	R	R	S	R	I	R	S	R	CAZ, CRX, CXM, AUG, AMX.
32	R	R	S	R	S	R	S	S	CAZ,GEN,CRX,AUG,CXM,AUG.
3	R	R	S	S	S	R	S	R	CAZ, CRX, AUG, AMX
% Resistance to Antibiotics	100%	100%	33%	67%	0%	100%	0%	67%	

Proteus vulgaris									
1	R	R	S	R	S	R	S	S	CAZ, CRX, CXM, AUG
2	R	S	R	I	S	R	R	I	CAZ, GEN, AUG, NIT
3	R	R	S	R	S	R	S	R	CAZ, CRX, CXM, AUG, AMX
% Resistance to Antibiotics	100%	67%	33%	67%	0%	100%	33%	33%	
Serratia marcensens									
1	R	R	S	R	S	R	R	R	CAZ, CRX, CXM, AUG, NIT, AMX
% Resistance to Antibiotics	100%	100%	0%	100%	0%	100%	100%	100%	

KEYS:

CAZ- Ceftazidime (30µg)	CRX- Cefuroxime (30µg)	GEN- Gentamycin (10µg)
AMX- Amoxilin (5µg)	OFL- Ofloxacin (5µg)	AUG- Augmentin (30µg)
NIT- Nitrofurantoin (30µg)	CTX- Cefotaxime (5µg)	
R- Resistant I- intermediate	S- Susceptible	

Table 4: Antibiotics Resistance Pattern of Gram Positive Bacteria from Water Samples

Test Organism	CAZ	CTR	GEN	CXC	OFL	CRX	ERY	AUG	Phenotype of Resistance Pattern
Staphylococcus aureus									
1	R	R	S	R	S	R	R	R	CAZ,CTX,CXC,CRX,ERY,AUG.
2	R	R	R	R	S	R	R	R	CAZ, GEN, CXC, ERY, AUG.
3	R	S	S	R	S	S	R	R	CAZ, CXC, OXA, AUG.
4	R	S	R	R	S	S	R	R	CAZ, GEN, CXC, ERY, AUG.
5	R	S	S	R	S	R	I	R	CAZ ,CXC, CRX, AUG.
6	R	S	R	S	S	R	S	R	CAZ ,GEN ,CRX, AUG.
7	R	S	S	R	S	S	R	R	CAZ, CXC. ERY ,AUG.
8	R	S	R	R	S	R	R	R	CAZ,GEN,CXC,CRX,ERY, AUG.
9	R	S	I	R	S	R	S	R	CAZ, CXC ,CRX,AUG.
10	R	S	S	R	S	S	R	R	CAZ,CXC,ERY ,AUG.
% Resistance to Antibiotics	100%	10%	40%	90%	0%	50%	70%	100%	
Klebsiella spp									
1	R	R	S	S	S	S	R	R	CAZ, CTX, ERY, AUG.
2	R	S	S	R	S	R	R	R	CAZ, CXC, CRX, ERY, AUG.
3	R	S	S	R	S	S	R	R	CAZ, CXC, OXA, AUG.
4	R	R	S	R	S	R	R	R	CAZ,CXC,CTX,CRX,ERY,AUG.
5	I	S	R	I	S	R	R	R	GEN ,CRX, OXA, AUG.
6	R	S	S	R	S	R	R	R	CAZ ,CXC,CRX,ERY, AUG.
7	R	S	S	R	S	R	I	R	CAZ, CXC. CRX ,AUG.
8	R	S	S	R	S	S	R	R	CAX, CXC, OXA, AUG.
% Resistance to Antibiotics	88%	25%	12%	75%	0%	63%	87%	100%	
Enterococcus faecalis									
1	R	S	R	R	S	S	R	R	CAZ,GEN,CXC,ERY,AUG.
2	R	R	S	R	S	R	R	R	CAZ,CTX,GEN,CXC,CRX,ERY,AUG.
3	R	S	S	S	R	S	R	R	CAZ, OFL, ERY, AUG.
4	S	S	S	R	S	R	R	R	COX, CRX, ERY, AUG.
5	R	R	S	I	S	S	R	R	CAZ,CTX,ERY,AUG.
6	R	S	S	R	S	S	R	R	CAZ ,CXC ,ERY, AUG.
7	R	S	S	R	S	R	R	R	CAZ, CXC. ERY ,AUG.
8	R	S	S	R	S	S	R	R	CAX, CXC, ERY, AUG.
9	S	S	S	R	S	R	R	R	COX ,CRX, ERY, AUG.
10	R	S	S	R	S	R	R	R	CAZ,CXC,CRX,ERY,AUG.
11	S	S	S	R	S	R	R	R	COX, CRX, ERY, AUG.
12	S	S	R	R	S	R	R	R	GEN,CXC,CRX,ERY,AUG.
13	R	R	S	R	S	R	S	S	CAZ, CTX, CXC, CRX.
14	R	S	S	R	S	S	R	R	CAZ ,CXC, ERY, AUG.
15	R	R	S	R	S	R	I	R	CAZ,CTX,CXC,CRX,AUG.
16	I	R	S	R	R	R	S	I	CTX, GEN, OFL, CRX.
17	R	R	S	R	S	R	R	R	CAZ,CTX,CXC,CRX,ERY, AUG.
18	S	S	R	S	R	R	S	R	GEN ,OFL, CRX,AUG.
19	R	R	S	R	S	R	S	R	CAZ,CTX,CXC,CRX, AUG.
20	R	R	R	R	S	S	S	R	CAZ,CTX, GEN,COX,AUG
21	R	S	R	R	S	R	S	R	CAZ,GEN,CXC,CRX AUG.
22	R	S	S	R	R	R	S	R	CAZ,CXC, OFL,CRX AUG.
23	R	S	S	R	S	R	S	R	CAZ, CXC, CRX, AUG.
24	R	S	S	S	S	R	R	R	CAZ, CRX, ERY, AUG.
25	S	R	S	S	S	R	R	R	CTX, CRX, OXA, AUG.
26	R	S	S	R	S	R	R	R	CAZ,CXC,CRX,ERY,AUG.
27	S	R	S	R	R	S	R	R	CTX,COX,OFL,ERY,AUG.
28	I	R	S	R	S	R	R	R	CTX,CXC,CRX,ERY,AUG.
29	S	R	S	R	S	R	S	R	CTX, CXC, CRX, AUG.
30	R	R	S	R	S	R	R	R	CAZ,CTX,COX,CRX, ERY,AUG.
% Resistance to antibiotics	73%	46%	16%	73%	13%	85%	66%	100%	

KEYS:

CAZ – Ceftazidime	GEN – Gentamicin	CXC - Cefotazine
ERY – Erythromycin	AUG –Augmentin	OFL - Ofloxacin
CRX –Cefuroxime	CTX -Ceftaxidine	

Table 5: Phenotypic Pattern of Multiple Antibiotic Resistance (MAR) Bacteria

No of Isolates	Antibiotics	Number of Occurrence Gram negative n=83(%)	Gram positive n=69(%)	Total Isolates (n=152)
	CAZ,CTX,CRX,AUG.	____	10(14.49%)	10(6.58%)
	CAZ,CTX,OXA,AUG	____	3(4.35%)	3(1.97%)
	CAZ,CRX,OXA,AUG	____	4(5.80%)	4(2.63%)
	CAZ,COX,OXA,AUG	____	4(5.80%)	4(2.63%)
	CAZ,COX,OXA,AUG	____	4(5.80%)	4(2.63%)
4	CAZ,CRX,GEN,AUG	2(2.41%)	____	2(1.32%)
	CAZ,GEN,CXM,AUG	3(3.61%)	____	3(0.02%)
	CAZ,CRX,CXM,AUG	5(6.02%)	____	5(3.29%)
	CAZ,CXM,NIT,AUG	3(3.61%)	____	3(0.02%)
	CXM,AUG,NIT,AMX	2(2.41%)	____	2(1.32%)
	CAZ,OFL,AUG,NIT	10(12.04)	____	10(7.92%)
	CAZ,COX,CRX,OXA,AUG	____	5(7.24%)	5(3.29%)
	CAZ,COX,CRX,OXA,AUG	____	2(2.89%)	2(1.32%)
	CAZ,CTX,CRX,OXA,AUG	____	2(2.89%)	2(1.32%)
	CAZ,CRX,CXM,AUG ,CPR	2(2.41%)	____	2(1.32%)
5	CAZ,CRX,CXM,NIT,AUG	5(6.02%)	____	5(3.89%)
	CAZ,CRX,CXM,AUG,CPR	6(7.235)	____	6(3.95%)
	CAZ,CRX,NIT,AUG,CPR	9(10.84%)	____	9(5.92%)
	CAZ,CRX,CXM,AUG,NIT	9(10.84%)	____	9(5.92%)
	CAZ, CRX, AUG, NIT, CPR.	9(10.84%)	____	9(5.92%)
	CAZ,CTX,COX,CRX,OXA, AUG.	____	4(5.8%)	4(2.63%)
	CAZ,CTX,CRX,COX, OXA,AUG	____	12(17.39%)	12(7.89%)
	CAZ,CRX,CXM,AUG,NIT, CPR,	9(10.84%)	____	9(5.92%)
6	CAZ,CRX,OFL,AUG,NIT,CPR	3(12.05%)	____	3(6.58%)
	CAZ,CRX,GEN,CXM,AUG,NIT	6(7.23%)	____	6(3.95%)
	CAZ,CRX.GEN,OFL,AUG,CPR	2(2.41%)	____	2(1.32%)
	CAZ,CXM,OFL,AUG,NIT,CPR	1(1.20%)	____	1(0.66%)
	CAZ,CTX,GEN,COX,CRX,OXA,AUG	____	3(4.35%)	3(1.97%)
7	CAZ,CTX,COX,OFL,CRX,OXA,AUG	____	4(5.80%)	4(2.63%)
	CAZ, CTX, CRX, GEN, AUG, NIT, AMX.	2(2.41%)	____	2(1.32%)

KEYS:
CAZ = Ceftazidine GEN = Gentamycin COX = Cloxacillin
AUG = Augmentin OFL = Ofloxacin CRX = Ceftaxidine
NIT = Nitrofurantoin CXM = Cefixime AMX = Ciprofloxacin
n= number of each of the isolates

Table 6: Plasmid Profile of Selected Multiple Antibiotics Resistance (MAR) Bacterial Isolated from Water

Isolates	Number of Plasmids (Kbp) n-34	Molecular weight of Plasmid
Escherichia coli 4	1	23.130
Escherichia coli 34	1	23.130
Pseudomonas aeruginosa 49	-	23.130
Bacillus megatarium 44	-	-
Staphylococcus aureus 42	1	-
Pseudomonas aeruginosa 22	1	23.130
Staphylococcus aureus 2	1	23.130
Klebsiella sp *13*	-	23.130
Bacillus cereus 43	-	-
Pseudomonas aeruginosa 5	-	-
Bacillus cereus 8	-	-
Enterobacter aerogenes 14	-	-
Klebsiela sp *18*	-	-
Serratia marcenscens 21	-	-
Escherichia coli 25	1	9.414
Staphylococcus aureus 27	-	-
Escherichia coli 29	2	23.130-5.64
Enterococcus faecalis 36	1	23.130
Bacillus cereus 38	-	-
Enterococcus faecalis 40	-	-
Enterobacter aerogenes 41	1	5.640
Enterococcus faecalis 45	1	23.130
Enterococcus faecalis 47	-	-
Enterobacter aerogenes 48	-	-
Proteus vulgaris 54	1	23.130
Bacillus cereus 56	-	-
Escherichia coli 59	1	23.130
Staphylococcus aureus 60	-	-
Enterobacter aerogenes 61	1	23.130
Proteus vulgaris 62	1	-
Staphylococcus aureus 10	1	23.130
Proteus vulgaris 35	1	5.640
Pseudomonas aeruginosa 32	1	9.416
Bacillus cereus 1	1	23.130
% Carrier of Plasmid	56%	

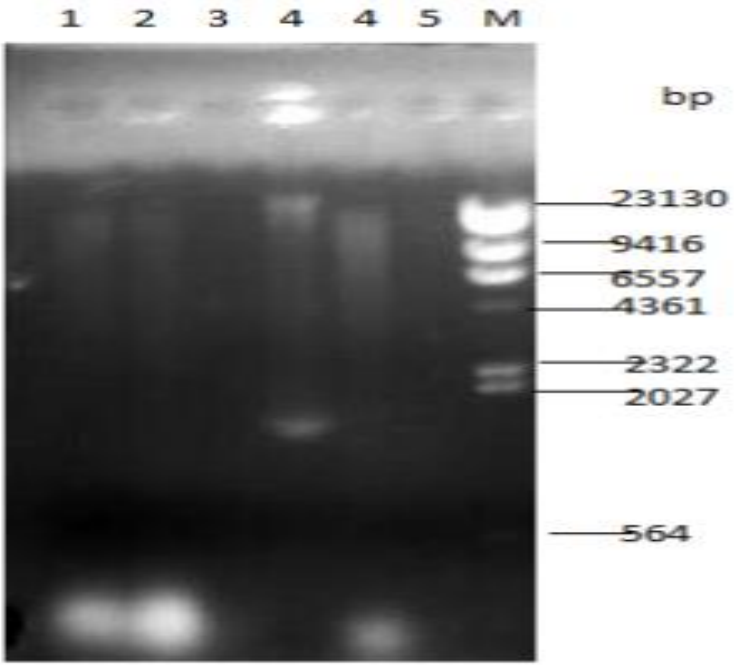

Fig 1: Plasmid Profile for Gram Positive Multiple Antibiotics Resistant Isolates.

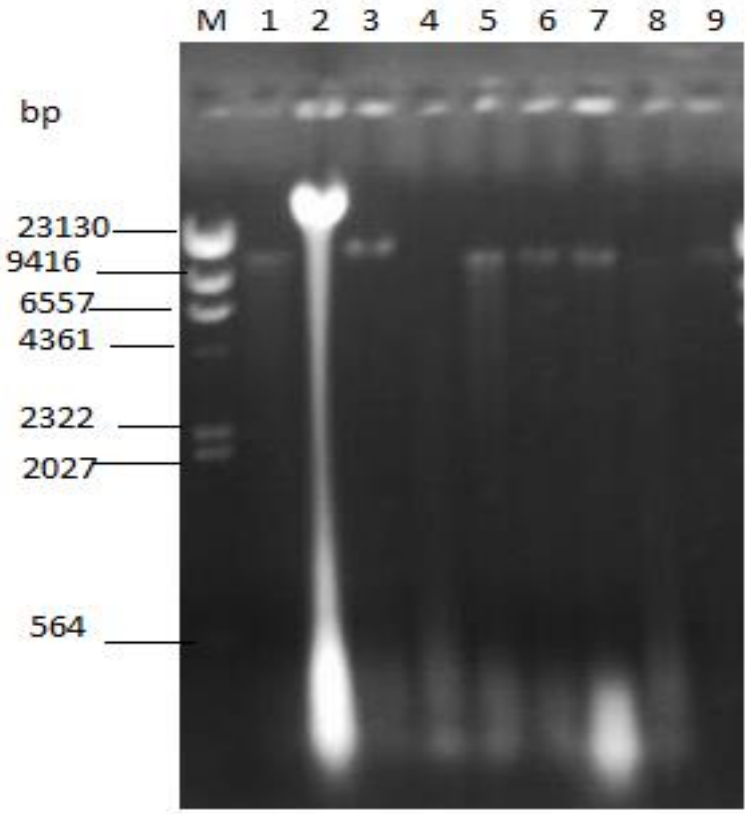

Fig. 2: Plasmid Profile for Gram Negative Multiple Antibiotics Resistant Isolates.

It was confirmed that *Pseudomonas aeruginosa* became susceptible to augmentin after been cured and *Staphylococcus aureus* also became susceptible to ceftriazole after curing while *E. coli* still maintained the earlier resistant pattern (Tables 7 and 8). Table 9 revealed the plasmid profiles of the multiple antibiotics resistant (MAR) bacteria selected for plasmid curing. This indicated the lost of plasmids in the three bacterial isolates after subjection to curing as depicted in figure 3.

The physiochemical results of the water samples ranged as follows: pH (6.5 - 6.80), colour (colourless), odor (odourless), taste (eusipid), temperature (25 – 27) ^{o}C, turbidity (0.010 - 0.028) NTU, total suspended solid (0.75 - 1.06), conductivity (0.000 - 0.030) mhos, dissolved oxygen (12.180 - 22.235) mg/L, biochemical oxygen demand (0.610 - 12.150) mg/L, acidity (10.10 - 13.000) mg/L, alkalinity (25.000 - 70.000) mg/L, hardness (22.30 - 60.000) mg/L. Nitrate (0.000 mg/L), Sulphate (10.000 - 12.000) mg/L, Chloride (15.000 - 76.000) mg/L, Magnesium (55.000 - 79.00) mg/L and Phosphate (0.000 mg/L). It was observed that chloride and magnesium were present in the samples.

Table 7: Antibiotic Resistance Pattern of Selected Bacteria Isolate from Water Sample

Isolates	Antibiotics											Phenotype of Resistance pattern
	CXC	ERY	AUG	NIT	CAZ	CRX	GEN	CTX	OFL	AMX	CTR	
Escherichia coli25	ND	ND	R	S	R	R	S	R	S	S	ND	CAZ,CRX,CTX,AUG
Pseudomonas aeruginosa32	ND	ND	R	S	R	R	S	R	S	S	ND	CAZ,CRX,CTX,AUG
Staphylococcus aureus2	R	R	R	ND	R	R	R	ND	S	ND	R	GEN,CRX,AUG,CXC,ERY,CTR,CAZ

KEYS:
CAZ - Ceftazidime
GEN - Gentamicin
CXC - Cefotazine
ERY - Erythromycin
AUG -Augmentin
OFL - Ofloxacin
CRX -Cefuroxime
CTX -Ceftaxidine
NIT - Nitrofurantoin
AMX - Amoxilin
CTR – Ceftriazole
I- Intermediate
R- Resistant
S- Susceptible
ND- Not determined

Table 8: Antibiotic Susceptibility Pattern of Selected Bacteria Isolated from Water Sample after Curing

Isolates	Antibiotics											Phenotype of Resistance pattern
	CXC	ERY	AUG	NIT	CAZ	CRX	GEN	CTX	OFL	AMX	CTR	
Escherichia coli25	ND	ND	R	S	R	R	S	R	S	S	ND	CAZ,CRX,CTX,AUG
Pseudomonas aeruginosa32	ND	ND	S	S	R	R	S	R	S	S	ND	CAZ,CRX,CTX,AUG
Staphylococcus aureus2	R	R	R	ND	R	R	R	ND	S	ND	R	GEN,CRX,AUG,CXC,ERY,CTR,CAZ

KEYS:
CAZ - Ceftazidime
GEN - Gentamicin
CXC - Cefotazine
ERY - Erythromycin
AUG -Augmentin
OFL - Ofloxacin
CRX -Cefuroxime
CTX -Ceftaxidine
NIT - Nitrofurantoin
AMX - Amoxilin
CTR – Ceftriazole
I- Intermediate
R- Resistant
S- Susceptible
ND- Not determined

Table 9: Plasmid Profile of MAR Bacteria Isolated from Water Sample after Curing

Isolates	Number of Plasmids (Kbp)	Molecular weight of Plasmid
Staphylococcus aureus2	-	-
Escherichia coli 25	-	-
Pseudomonas auriginosa 32	-	-

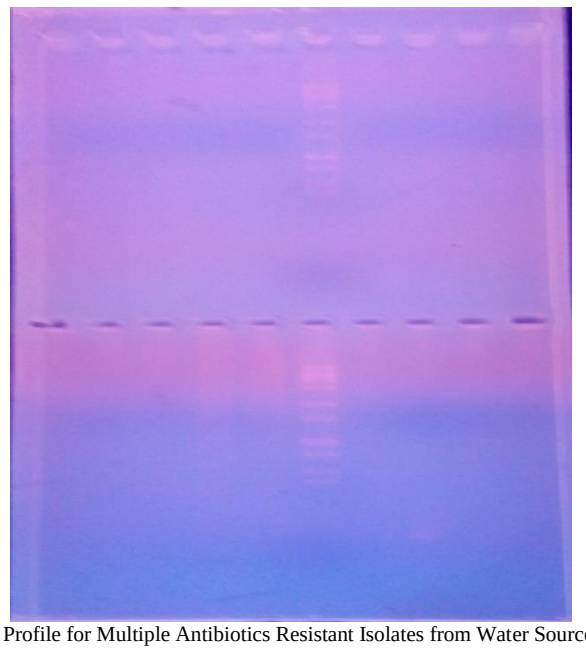

Fig. 3: Plasmid Profile for Multiple Antibiotics Resistant Isolates from Water Sources after Curing.

Table 10: Physicochemical Properties of Water Sample

PARAMETERS	Results					
	A	B	C	D	E	F
Temperature (ºC)	27.0	26.2	25.3	25.0	26.2	26.00
pH	6.5	6.7	6.6	6.80	6.5	6.80
Conductivity (NH/CM)	0.03	0.02	0.01	0.00	0.02	0.03
Turbidity (NTU)	0.028	0.000	0.000	0.000	0.010	0.020
Dissolved oxygen	22.235	15.180	16.100	15.180	18.000	12.180
Total hardness (mg/l)	25.00	22.3	40.00	25.00	50.00	60.00
Total alkalinity	35.00	25.00	27.00	35.00	25.00	70.00
Colour	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless
Odour	Odourless	Odourless	Odourless	Odourless	Odourless	Odourless
Nitrate (mg/l)	0.00	0.00	0.00	0.00	0.00	0.00
Sulphate (mg/l)	12.000	11.000	10.000	11.000	10.000	11.000
Chloride (mg/l)	15.00	55.00	76.00	26.00	20.00	25.00
Phosphate (mg/l)	0.00	0.00	0.00	0.00	0.00	0.00
Magnesium (mg/l)	77.00	56.00	64.00	79.00	55.00	77.00

KEYS:
A - Aba Ola Bore Hole (Ekiti East Local Government Area)
B - Eporo Bore Hole (Ekiti South West Local Government Area)
C - Kajola Well (Emure Local Government Area)
D - Ogotun Bore Hole (Gbonyin Local Government Area)
E - Oke Ikere Well (Ikere Local Government Area)
F - Igbara Odo stream (Ise/Orun Local Government Area)

4. Discussion

Water is natural resources that are very essential to life and other living things. It is useful in various aspect of life such as: in cooking, agricultural practices and drinking. Ground water has a unique features compare to other sources of water which render them suitable for public supply (Alexandra, 2008). The quality of water is determined by bacteriological, physiochemical and mineral analysis (Makinde and Akande, 2012). The bacteriological analysis of the water samples showed the extent to which the water was contaminated by various microorganisms, most especially coliform bacteria which could be attributed to contamination with faeces of either human or animal origin or as a result of inadequately treated sewage discharge (WHO, 2006).

The mean of the total bacterial, total coliform and total enterococcal counts of the water samples in this present study were higher than the specified limit advocated by WHO standard (2006). This might be poor hygiene and sanitary conditions such as clothe and dishwashing as well as defecating in and near the water bodies, coupled with the location of the water considering the bushes and shrubs around the water bodies; which could serve as route for possible contaminations (Okonko *et al*., 2008). This is in accordance with the report of Edama *et al*. (2001) which explains that the presence of bushes and shrubs around water bodies makes it likely and possible of some individuals (man or animal) which may come around to drink water thereafter defecate in or around the surface water. Furthermore, microbial contamination of drinkable water such as underground water according to Roohul *et al*., (2012), may be attributed to leakage in pipes; cross contamination with wastewater; poorly constructed well head; short distance between water supply network and sewage supply; construction of septic tanks near wells and drinking water supply lines; run-offs; infiltration of wastes and direct deposition of waste water through leakage. This evident the high microbial load encountered for the well and bore-hole water analyzed in present study.

Majority of the bacteria species that were isolated in this study were identified to be same as those commonly encountered in water and aquatic environments as reported by Nafaida *et al*., (2006) and Nicholas *et al*., (2009). They were detected to be members of coliforms, which are Gram negative, facultative anaerobes and non-spore formers that ferment lactose within 48 hours (Prescott *et al*., 2008). The high number of bacteria from the family of Enterobacteriaceae and coliform bacteria is an indication that the water samples are not portable and thus unfit for domestic use (WHO, 2006).

The isolation of *Escherichia coli* from the water samples correlates with the past studies that have presented *Escherichia coli* as a common encounter in different water sources such as rivers, streams, rain water, well water, underground water and even pipe borne water (EPA, 2002). The correlation of this study with previous study is making it seem like *Escherichia coli* is a normal flora of water bodies and can be isolated from any water body as earlier reported by Zamxaka *et al*. (2004). The implications of this organism in water and food related pathogenic infections have been reported by different researcher (Wastesan *et al*., 2001; Kaper *et al*., 2004).

All the bacteria isolated during this investigation have however been reported by Cheesbrough (2004) as potential pathogens. Following the report of Yagoub and Ahmed (2010), Pseudomonas auruginosa and other potentially pathogenic bacteria is significant enough to admit that the quality of these water sources has been adversely deteriorated thereby subjecting the immune-compromised individual in the community patients to greater health risks. Going by the description of Schlegel (2002), Enterobacter aerogenes isolated from these water samples are regarded as non-fecal coliforms mostly found in vegetation and soil; this further explains how the bushes and shrubs around the water may have contributed to the contamination. It becomes more concerned to detect that the set of bacterial isolates in this study were similar to those documented to proliferate in leachate samples as reported by Odeyemi *et al*. (2011). This however could be traceable to the proximity of these water bodies to dumpsites, through which these contaminant may have find their ways into the water via percolation, seepage or run-off; as narrated by the report of Odeyemi *et al*. (2012).

Antibiotic sensitivity results shows that majority or almost all the bacteria isolated were resistant to the various antibiotics used. This is in support with the report of Odeyemi *et al*. (2010) which stated that up to about 80% of the coliform found in the ground water is resistant to antibiotics. The mechanisms used by these organisms include: modification of the target site, change in bacteria cell membrane, production of enzymes which inactivates the drug, reduction in the cellular uptake of drugs and rapid extrusion of the antibiotics. These mechanisms arise when bacteria are subjected to genetic changes as a result of mutation or by acquisition of a new genetic material (Prescott *et al*., 2008).

The high rate of antibiotics resistance by the *Escherichia coli* isolates in this study correlates with the work of Odeyemi *et al*. (2013) which reported the resistance of *Escherichia coli* to about seven of the eight antibiotics used. The multiple resistance pattern of the *Escherichia coli* isolates as shown in the antibiotics testing also agrees with the findings of Heike and Reinhard (2005); Walsh *et al*. (2005) which also reported the growing discoveries of antibiotics resistant strains and attributed this to the use of antibiotics in animal husbandry which has caused genotypic change due to chromosomal mutation. Some microorganisms that are found in the soil find their way into the water bodies through the surface run off and because many of these soil microorganisms have the

ability to produce antibiotics normally, they acquire some mechanisms that can render these antibiotics ineffective hence, they have no effects on them when they are used (Akonai, 2003). Another way by which these isolated bacteria can develop resistance to the various antibiotics is through the transfer of antibiotic resistant gene from one organism to another.

Three isolate (*E. coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) were selected among the plasmid mediated bacteria for curing after been subjected to antibiotic sensitivity test with the aim curing the plasmids contained in the bacteria according to the method of Brown (2000). After been subjected to curing, *Pseudomonas aeruginosa* that exhibited plasmid resistance ceftazidine (CAZ), cefuroxime (CRX), Augmentin (AUG) and cefotaxime (CTX) became susceptible to Augmentin (AUG) after the plasmid has been cured, but still maintain resistant ability against ceftazidine (CAZ), cefuroxime (CRX), and cefotaxime (CTX). *Staphylococcus aureus* that was resistant to gentamicin (GEN), cefuroxime (CRX), augmentin (AUG), cefotazine (CXC), erythromycin (ERY), ceftazidine (CAZ) and ceftriazole (CTR) also became susceptible to ceftriazole (CTR) after been cured. *E. coli*25 that exhibited plasmid resistance to ceftazidine (CAZ), cefuroxime (CRX), cefotaxime (CTX), and augmentin (AUG) still remained unchanged even after the plasmid has been cured, this could be as a result of mutation or acquisition of new genes.

The resistant pattern of the isolates (*E. coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) was confirmed by subjecting the cured isolate to antibiotic susceptibility testing using the same antibiotics. The three (3) bacteria isolates (*E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) had no plasmid recovered after been subjected to curing which indicate that plasmid has been lost during the process of curing as described by (Vivyan *et al*., 1972).

5. Conclusion and recommendations

This present study revealed that the sources of water in the Ekiti South Senatorial district are not safe for drinking especially because of the incidence and abundance of MAR bacteria in water sources. Serious health hazards could result from consumption of such water. Hence, proper and adequate treatment of this water is highly required. Human attitudes such as dumping of refuse or untreated sewage and defecating in and around water bodies should also be discouraged

This study recommends the provision of portable water, modern sanitary sewage disposal facilities and creation of awareness to the people in the community of the risk associated with the consumption of contaminated water. Also, sewage and refuse should not be dumped into the stream water around the landfill site in order not to increase the nutrient availability of the water which will aid growth of microbes in water bodies. Further molecular characterization of the isolates is recommended to ascertain identity and other genetic factors that determined the multiple resistances of the microbes

References

[1] Figueras, M. J. and Borrego, J. J. (2010). New perspectives in monitoring drinking water microbial quality. *Int. J. Environ. Res. Public Health* 7, 4179–4202. https://doi.org/10.3390/ijerph7124179.

[2] Birnboim, H. C. and Doly, J. (1979). A rapid alkaline procedure for screening recombinant Plasmid DNA. Nucleic Acid Res. 7: 1513-1523. https://doi.org/10.1093/nar/7.6.1513.

[3] Kado, C. I and Liu, S. T. (1981). Rapid procedure for detection and isolation of large and small plasmids. *J. Bacteriol*. 145: 1365-1373.

[4] Datta, N., Hadges, R. W., Shaw, E. J., Sykes, R. B. and Richman, M. H. (1971). Properties of an R- factor from *Pseudomonas aeruginosa. J. Bacteriol*. 108: 1244-1249.

[5] Brown, T. A. (2000). Essential Molecular Biology, A Practical Approach. Oxford University Press. 2nd Edition. Pp 69-102.

[6] APHA (1985). Standard Methods for Examination of Water and Wastewater, 20th Edition, American Public Health Association, Washington D. C.

[7] APHA (1998). Standard Methods for the Examination of Water and Wastewater, 20th edition. American Public Health Association, Washington, D.C.

[8] Trivedy, R. K.and Goel, P. K (1986). Chemical and biological methods for water pollution studies, Environmental Publication, Karad, Maharashtra.

[9] Akonai, A. K., Lamikanra, A. and Ola, J. B (2003) Transmissible thrimethoprim resistance of strains of *Escherichia coli*. From cases of infantile diarrhea. *Journal* of *medical microbiology*. 32(7): 159-162.

[10] Alexandra, P. (2008). Evaluation of Ground water quality of Mobi town in Adamawa state, Nigeria.*Afr Journal of Biotech*, 7:1712-1715. https://doi.org/10.5897/AJB08.227.

[11] Brown, T. A (2010). "Chapter 2 – Vectors for Gene Cloning: Plasmids and Bacteriophages". Gene Cloning and DNA Analysis: An Introduction. *Wiley-Blackwell*. 10(1): 17-18.

[12] Cheesbrough, M. (2004). District Labor-atory Practice in Tropical Countries (part ii). Cambridge University Press. Pp 50-150.

[13] CLSI (2005). Performance standards for antimicrobial susceptibility testing; fifteenth informational supplement, Clinical and Laboratory Standard Institute Wayne, Pa. M100-S15, 25(1): 13-17.

[14] Edama, M. D., Omemu, A. M and Fapetu, O. M (2001). Microbiological and Physiochemical Analysis of Different Sources of Drinking Water in Abeokuta, Nigeria. *Niger. J. Microbial*, 15(1): 57-61

[15] Environmental Protection Agency (2002). Drinking Water, National Primary Drinking Water Regulations: Total Coliforms (including fecal and *E.coli*). *Fed.Regist*, 54(124): 27544.

[16] Heike, V. and Reinhard, M. (2005). Antimicrobial Resistance of *Escherichia coli* and Therapeutic Implications. *Int. J. Med. Microbiol.* 295(6-7): 503-511. https://doi.org/10.1016/j.ijmm.2005.07.002.

[17] Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. and Williams, S. T. (1994). Bergey's manual of determinative bacterial. Baltimore, Ninth Edition, Williams and Wilkins, London, UK, 787.

[18] Kaper, D. M. and Roane, T. M. (2004). Differential responses of a mine tailings Pseudomonas isolate to cadmium and lead exposures. Biodegradation, 17 (4), 379-387.

[19] Makinde, A. M. and Akande, F. I. (2012). Effects of Lead and Simulated Acid Rain on Chlorophyll Contents of Selected Trophical Mosses. *Ife Journal of Science*, 14(2):309-313.

[20] Nicholas, E. J., Andrew, R. P. and James, M. B. (2009). Evaluation of a Simple Method to Classify the Thermal Characteristics of Streams using a Nomogram of Daily Maximum Air and Water Temperatures. *North American Journal of Fisheries Management*, 29:1605-1619. https://doi.org/10.1577/M08-251.1.

[21] Odeyemi, A. T. and Agunbiade, R. O. (2012). Bacteriological and metal analyses of Water samples from Awotunde fish pond and river. *Scientific Journal of Microbiology*, 1(2): 48-54.

[22] Odeyemi, A. T., Ajayi, A. O. and Igbalajobi, O. A (2013). Plasmid profile of isolated bacteria from Arinta water fall in Ipole-iloro, Ekiti State. *Journal of microbiology research*, 3(1):32-38.

[23] Odeyemi, A. T., Dada, A. C., Ogunbanjo, O. R. and Ojo, M. A. (2010). Bacteriological, physiochemical and mineral studies on Awedele spring water and soil samples in Ado-Ekiti, Nigeria. *African Journal of Environmental Science and Technology*, 4(6):319-327. https://doi.org/10.5897/AJEST09.194.

[24] Odeyemi, A. T., Faweya, E. B., Agunbiade, O. R. and Ayeni, S. K. (2011). Bacteriological, Mineral and Radioactive Contents of Leachate Samples from Dump Sites of Ekiti State Government Destitute Center in Ado Ekiti. *Archives of Applied Science Research*, 3(4): 92-108.

[25] Okonko, I. O., Adejoye, O. D., Ogunnusi, T. A., Fajobi, E. A. and Shittu, O. B. (2008). Microbiological and Physiochemical Analysis of Different Water Samples used for Domestic purposes in Abeokuta and Ojata, Lagos State, Nigeria. *African Journal of Biotechnology*, 7(5):617-621.

[26] Olutiola, P. O., Famurewa, O. and Sonntag, H. G. (2001). An introduction to General Microbiology. A Practical Approach. Cad Heideberg Virlag Sanstaltund Druckerei Gmbh Helderberg, Germany, pp. 38-51.

[27] Prescott, M. L., Harley, P. J. and Klein, A. D. (2008). Microbiology. 8th Edition. McGraw Hill Companies Inc. New York.

[28] Roohul, A., Syed, S. A., Zubair, A. and Jabar, Z. K. (2012). Microbial Analysis of Drinking Water and Water Distribution System in New Urban, Peshawar. *Current Research Journal of Biological Science*, 4(6): 731-737.

[29] Schlegel, H. G. (2002). General Microbiology. 7th Ed. Cambridge University Press. 480p.

[30] Vivyan, E., Hedges, R. W. and Datta, N. (1972). Two modes of curing transmissible bacterial plasmids. *J. Gen. Microbiol*., 70, 443 - 452. https://doi.org/10.1099/00221287-70-3-443.

[31] Walsh, C., Duffy, G., O'mahony, R., Faning, S., Blain, I. S. and McDowell, D. A. (2005). Antimicrobial Resistance in Irish Isolates of Verocytotoxigenic *Escherichia coli* VTEC. *Int. J. Food Microbiol*, 109(3): 173-178. https://doi.org/10.1016/j.ijfoodmicro.2006.01.023.

[32] Wasteson, Y., Garvey, P., McDowell, D. A., Cola, J. and Duffy, G. (2001). Control of Verocytogenic *E.coli*. Special edition of *Int. J. Food Microbiol*. 66: 1-2.

[33] World Health Organization (WHO), (2006). Guidelines for Drinking Water Quality Vol. 1 Geneva, Switzerland.

[34] Yagoub, A. E. A. and Ahmed, T. A (2010). Microbiological evaluation of the quality of tap water distributed at Khartoum state. *Science alert.*

[35] Zamxaka, M., Pironcheva, G. and Zamxaka, N. Y. O. (2004). Bacterial Community Patterns of Domestic Water Sources in the Gogogo and Nkonkobe Areas of the Eastern Cape Province, South Africa. *Water SA*, 30(3): 341-346. https://doi.org/10.4314/wsa.v30i3.5082.